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13. ABSTRACT *(Maximum 200 words)*

Immobilized enzymes are used in bioreactors and biosensors. Current methods for immobilizing enzymes include adsorption or covalent attachment to a support, microencapsulation and entrapment within a membrane/film or gel. The ideal enzyme immobilization method would 1) Employ mild chemical conditions; 2) Allow for large quantities of enzyme to be immobilized; 3) Provide a large surface area for enzyme/substrate contact within a small total volume; 4) Minimize barriers to mass transport of substrate and product and 5) Provide a chemically and mechanically robust system. This report describes a new method for enzyme immobilization that satisfies all of these criteria. We have developed a template-based synthetic method that yields hollow polymeric microcapsules of uniform diameter and length. These microcapsules are arranged in a high density array in which the individual capsules protrude from a surface like the bristles of a brush. We have developed procedures for filling these microcapsules with high concentrations of enzymes. The enzyme-loaded microcapsule arrays function as enzymatic bioreactors in both aqueous solution and organic solvents.

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Enzyme immobilization, microcapsules, polypyrrole

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POLYMERIC MICROCAPSULE ARRAYS -

SYNTHESIS AND APPLICATIONS

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1. Introduction:

Over the past few years, enzyme immobilization has emerged as an important method for the development of biosensors and bioreactors.[1- 10] Although a number of enzyme immobilization methods are available, no ideal general method for immobilization has yet been developed. Current methods for enzyme immobilization include adsorption or covalent attachment to a support, microencapsulation, and entrapment within a membrane/film or gel.[1,4] These methods often suffer from several disadvantages. Covalent binding or cross-linking methods often expose the enzyme to harsh synthesis conditions. This may result in the loss of activity of the enzyme.[2] On the other hand, immobilization of enzymes by adsorption can be achieved under mild conditions. In these cases, however, leakage of the enzyme from the carrier may occur on changes of pH or ionic strength.[2] In the case of entrapment methods, diffusional constraints have to be overcome.[12,13,15]

We have used a novel microencapsulation approach for the immobilization of enzymes. This approach is of a very general nature and can be used to immobilize nearly any kind of enzyme.This entails "template synthesis" [11-14] of polymeric microcapsules (of uniform diameter and length) and immobilization of the desired enzyme within these microcapsules. The microcapsules are arranged in a high-density array in which the individual capsules protrude from a surface like the bristles of a brush.The enzyme-loaded microcapsule arrays function as enzymatic bioreactors in both aqueous solution and organic solvents.

2. Enzyme-immobilization procedure:

The template-based synthetic method [21- 24] entails synthesis of the capsules within the pores of a microporous membrane. The microporous membrane used for this study contained $1x10^8$ pores /cm^2 and the pores were 400 nm in diameter. The thickness of the membrane was 10 μm.

Microcapsule arrays were produced using a combination of

electrochemical and chemical methods.[15] The surface of the template membrane is first sputtered with a 50 nm layer of gold (Fig. 1a) .The gold layer acts as an electrode for the electrochemical polymerization of pyrrole. Polypyrrole coats the electrode surface and short (1μm) polypyrrole 'plugs' are also deposited within the pores (Fig 1b). The next step is the chemical polymerization of polypyrrole microtubules (Fig1c).[21-23] The electrochemically polymerized plugs become caps for the chemically polymerized tubules. The capped tubules are then filled with the desired enzyme by vacuum filtration of a solution of the enzyme through the capsule-containing membrane (Fig 1d). Water molecules pervaporate through the polypyrrole plug whereas the much larger enzyme molecules are retained within the capsules. After filtration of the enzyme, torr seal epoxy is applied to the open end of the capsules (Fig 1e). After curing of the epoxy, the entire assembly is immersed in methylene chloride to dissolve the membrane. This yields the desired array of enzyme-loaded microcapsules.

Scanning electron microscopic images of the microcapsule array were taken. The unique brush-like arrangement of these capsules was observed. The exposed capsule surface area is high (ca. 12 cm^2 of capsule surface area per cm^2 of substrate epoxy surface area). A transmission electron microscopic image of capsules that had not been attached to the epoxy surface is shown in figure 2. TEM revealed 1 μm caps and extremely thin-walled capsules (ca. 25nm thick).

3. Encapsulated enzymes:

Five enzymes have been encapsulated using this method. These are catalase, glucose oxidase, subtilisin, trypsin and alcohol dehydrogenase. Standard assay methods were used to show that the encapsulated enzyme retains its catalytic activity. A typical example of an enzyme system that we have studied is glucose oxidase. Encapsulated glucose oxidase (GOD) was used to catalyse the two electron two proton oxidation of glucose. The standard o-dianisidine /peroxidase assay was used to study this reaction.[17] When the array of glucose oxidase-loaded microcapsules is immersed in a solution containing glucose, o-dianisidine and peroxidase, a red colour develops as a result of the oxidation of dianisidine (Fig3). Curves 'a' and ' b' in figure 3 compare

catalytic activities for microcapsule arrays containing two different loading levels of glucose oxidase. As would be expected, the capsules with the higher glucose oxidase content show higher enzyme activity. The ability to control the amount of enzyme immobilized is an important feature of this microcapsule immobilization method. An assay of the quantity of enzyme loaded into the capsules used for curve 'a' showed that an amount equivalent to 625 mg glucose oxidase per ml of capsule volume was present in each capsule. It can be shown from the specific volume of glucose oxidase that this quantity occupies 47% of the available volume within a microcapsule.

Enzyme immobilization is a critical issue in the development of new biosensors. A number of proposed glucose sensors have been prepared by physically entrapping glucose oxidase within the polypyrrole films.[5-10] Curves 'c' and 'd' in Figure 3 show that the enzymatic activities for two such polypyrrole films. The films were ~4.7μm (c) and 0.8 μm (d) thick.(The activities for these films are nearly the same because only a thin layer (~0.3μm thick) at the outer surface of the polypyrrole film is enzymatically active.) [7,9] A comparison of the slopes of curves 'c' and 'd' with the slope of curve 'a' clearly shows that higher enzymatic activity can be achieved with our microcapsule-immobilization method. Hence, our microcapsule arrays show promise for the development of new types of enzymatic biosensors.

We have shown that polymeric microcapsule arrays can be prepared via the template synthesis method and that these arrays provide a general route for enzyme immobilization. The advantages of this method are that mild conditions are employed , high loadings of enzyme are achieved , a large surface area for enzyme-substrate contact within a small total volume is made possible , barriers to mass transport of substrate and product through the thin walls of the capsule are minimized and a chemically and mechanically robust system was provided.We are currently attempting to fabricate a biosensor using this concept of immobilization.

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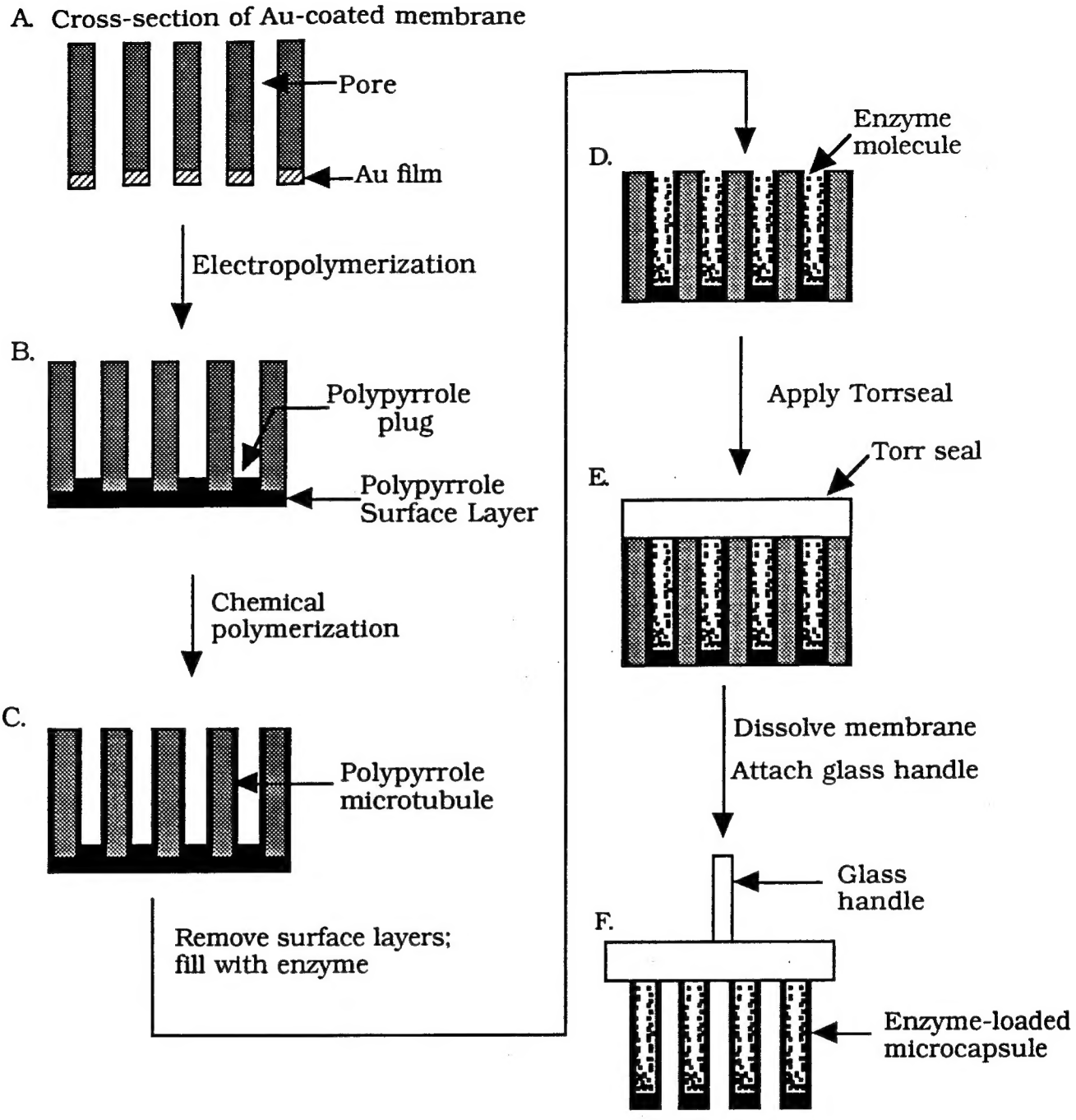
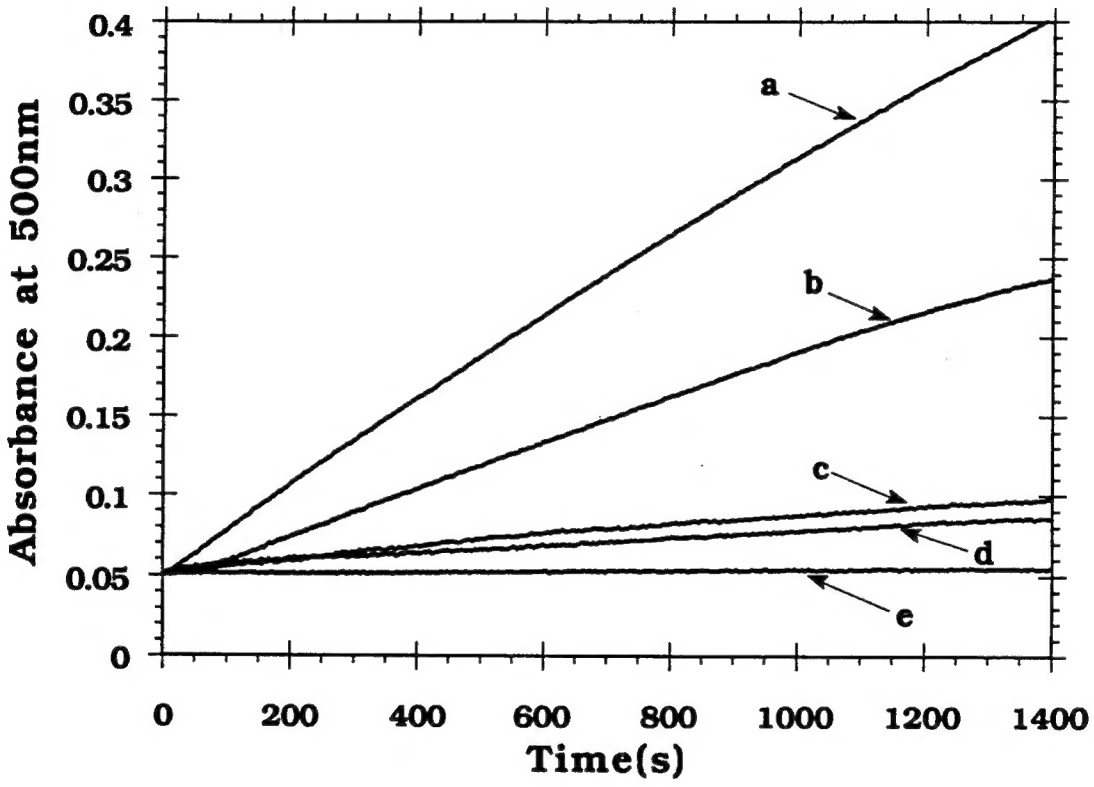
Figure Captions:

Fig. 1. Schematic diagram of methods used to synthesize and enzyme-load the microcapsule arrays.

Fig. 2. Transmission electron micrograph of microcapsules that had not been attached to the epoxy surface. Scale bar in upper left corner of B is 1.0 μm.

Fig. 3. Absorbance for the oxidized form of o-dianisidine after immersion of glucose oxidase-loaded and empty microcapsule arrays into a glucose oxidase assay solution (see text).


A. Cross-section of Au-coated membrane
Pore
Au film
Electropolymerization
B.
Polypyrrole plug
Polypyrrole Surface Layer
Chemical polymerization
C.
Polypyrrole microtubule
Remove surface layers; fill with enzyme
D.
Enzyme molecule
Apply Torrseal
E.
Torr seal
Dissolve membrane
Attach glass handle
F.
Glass handle
Enzyme-loaded microcapsule


Absorbance at 500nm
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
200
400
600
800
1000
1200
1400
Time(s)
a
b
c
d
e